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Electrical changes produced by injury to the rat myocardium *in vitro* and the protective effects of certain antiarrhythmic drugs

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1 Glass microelectrodes were used to record intracellular electrical activity from rat isolated and superfused atrial myocardium during external electrical stimulation.

2 After 2 h in normal oxygenated physiological salt solution the muscle was exposed for 30 min to a superfusate simulating the composition of extracellular fluid during myocardial ischaemia (SI). This fluid contained lactate (20 mM), a raised potassium concentration (7 mM), no glucose and a pH lowered to 6.4, and was gassed with N_2 in place of O_2 (hypoxia).

3 During SI the diastolic threshold voltage for stimulation increased, the speed of action potential conduction between the right and left atria slowed, and both the effective and functional refractory periods of the right atrium shortened, as did the duration of the right atrial action potential.

4 The only component of SI which separately caused electrical changes similar to those of the full simulation was hypoxia.

5 Addition to the superfusate of verapamil ($0.5\,\mu g\,ml^{-1}$), sulphinpyrazone ($1-20\,\mu g\,ml^{-1}$) or indomethacin ($10-20\,\mu g\,ml^{-1}$) attenuated many of the SI-induced electrical changes, although indomethacin was much less effective than the other two drugs.

6 Lowering the calcium concentration of the superfusate from 2 mM to 0.5 mM protected against the SI-induced electrical changes that were inhibitable with sulphinpyrazone and verapamil.

Introduction

During myocardial ischaemia, in several mammalian species, the extracellular concentrations of hydrogen and potassium ions rise, the availability of glucose and oxygen declines, while lactate accumulates. Moreover, these extracellular chemical changes, particularly in combination, tend to shorten the duration of myocardial action potentials and their associated refractory periods (Downar *et al.*, 1977; Morena *et al.*, 1980; Cobb *et al.*, 1985; Ferrier *et al.*, 1985) and tend to slow the rate of conduction (ROC) of myocardial action potentials (Nakaya *et al.*, 1980; 1981; 1982; Kimura *et al.*, 1982; 1983; Gettes *et al.*, 1985). These electrical disturbances are arrhythmogenic, particularly when they occur heterogeneously throughout the myocardium (Northover, 1986). The present experiments were designed to explore the extent to which these electrical changes, produced by the chemical abnormalities that characterize the myocardium during ischaemia, were able to be created in the rat atrium *in vitro*, and the extent to which they were able to be modified by certain antiarrhythmic drugs.

Methods

Rats of the Sprague-Dawley strain weighing 340–480 g were killed by a blow to the head. The heart was removed quickly, the atria separated from the ventricles and attached with the endocardial surface upwards to the base of a superfusion trough maintained at 34°C. The muscle was exposed, unless specified otherwise, to a normal superfusate (NS) of the following composition (mM): NaCl 138, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.5, $NaHCO_3$ 10 and glucose 10, and gassed with a mixture of 95% O_2 plus 5% CO_2, giving a pH of 7.3.

Transmembrane potentials were recorded from subendocardial muscle fibres by means of a pair of glass microelectrodes filled with a 3 M solution of KCl and having resistances of $1-2 \times 10^7\,\Omega$. One microelectrode was lowered vertically by means of a micromanipulator towards the endocardial surface of the right atrium. Voltages detected by this microelectrode were passed by a single-ended high input-impedence cou-

pler with facilities for capacitance neutralisation (Type 8124, made by C.F. Palmer) to both an oscilloscope and a transient store microprocessor (Type 140, made by Bioscience). Stored signals were able to be replayed from the latter device at speeds of up to 2,000 fold slower than those at which they were recorded, permitting action potentials to be printed without distortion on paper, using a conventional pen recorder and allowing the maximum rate of depolarization (MRD) during the upstroke of the action potential to be found. The muscle was stimulated throughout an experiment at 4 Hz via a pair of platinum wire electrodes placed on the right atrial appendage. Square wave pulses of current, each 2 ms in duration and isolated from earth were used at a voltage of twice the prevailing diastolic threshold (DT). The effective refractory period (ERP) of right atrial muscle was determined using paired stimuli, and was taken as the interval between the most closely spaced pair of stimuli both of which yielded action potentials. The interval separating the upstrokes of the pair of action potentials so obtained was taken as the functional refractory period (FRP). The rate of conduction (ROC) of action potentials between right and left atria was measured with the aid of the second glass microelectrode, which was inserted in the wall of the left atrium. Voltage signals from both microelectrodes were displayed on a dual channel oscilloscope. Knowing the distance between the tips of the two microelectrodes and the time interval between the upstrokes of the action potentials recorded from them, it was possible to calculate the apparent ROC. However, since action potentials may not have conducted via the most direct route between the two microelectrodes, the ROC values may have been underestimated.

At the start of each experiment a tissue was allowed to equilibrate in NS for 2 h after which time control values of right atrial action potential duration measured at 60% repolarization (APD), DT, MRD, ERP and FRP were determined, together with the ROC between right and left atria. The superfusate was then changed for 30 min to one of abnormal composition, simulating the extracellular fluid composition of ischaemic muscle in one or more respects, as suggested by Ferrier *et al.* (1985). The relevant compositional abnormalities of this fluid were a high KCl concentration (7 mM), the presence of sodium lactate (20 mM), the absence of glucose, a pH lowered to 6.4 by lowering the $NaHCO_3$ concentration to 4 mM, and hypoxia produced by gassing with N_2 in place of O_2, giving a Po_2 in the superfusate of < 25 mmHg. When all these abnormalities were present in combination it was considered that conditions of simulated ischaemia (SI) prevailed.

Verapamil hydrochloride (supplied by Abbott Laboratories) was dissolved in distilled water. Indomethacin (supplied by Merck Sharp and Dohme, Ltd) and sulphinpyrazone (supplied by Geigy Pharmaceuticals, Ltd) were converted to their sodium salts with the aid of an equivalent weight of Na_2CO_3 and then prepared as aqueous solutions at pH 7.5. Aliquots of concentrated drug solutions were added to the superfusate just before use.

Results

Simulated ischaemia and its components

Exposure of the myocardium to SI caused the DT to rise, maximum values being reached after 15–20 min. Thereafter the DT values remained substantially unchanged for the remainder of the 30 min period of testing (Figure 1). Coincident with the rise in DT there was a progressive slowing of the ROC and a shortening of APD (Table 1), ERP and FRP (Figure 1). Shortening of refractory periods was most marked 12–15 min after the start of exposure to SI. Thereafter, refractory periods rose slowly (Figure 1), although APD values showed no tendency to increase, suggesting the development of some post-repolarization refractoriness. In all preparations total failure of conduction of action potentials from the right to the left atrium occurred within the 30 min period of SI. The mean time required to produce total right to left atrial conduction block was 18 min. Despite this, the right atrium continued to generate action potentials throughout the 30 min period of SI provided that the stimulus voltage continued to be adjusted to twice the prevailing DT. During exposure to NS the MRD values were acceptably consistent both between different preparations and between different places in the atrial wall of any given preparation. Unfortunately, during exposure to SI, although MRD values always tended to decrease, the decrease was extremely variable, not only between different preparations but also even at different places within a single preparation. For this reason MRD values have not been presented.

Experiments were performed to identify which components of SI were responsible for the observed electrical changes. In the first series of such experiments each component of SI was tested separately in an otherwise normal superfusate. Hypoxia was the only component of SI which shortened refractory periods, raised the DT and slowed the ROC when tested alone in an otherwise normal superfusate (Figure 2). These changes were not as great, however, as those produced by full SI (Figure 2). In the second series of experiments superfusates containing all but one of the components of SI in turn were tested. Omission of single components produced smaller rises in DT, less slowing of the ROC and less shortening of the refractory periods than was produced by exposure to the full SI (Figure 2). Exceptions to this general

Table 1 The ability of sulphinpyrazone, indomethacin and verapamil to inhibit simulated ischaemia (SI)-induced shortening of the right atrial action potential duration and SI-induced slowing of the conduction of action potentials between the atria

Superfusate	*Drug*	*Concentration* ($\mu g\ ml^{-1}$)	*APD* (ms) *10 min*	*15 min*	*20 min*	*ROC* ($cm\ s^{-1}$) *5 min*	*10 min*	*15 min*	*Inter-atrial block* (min)†
NS	–	–	18.1 ± 2.3	18.2 ± 2.2	19.2 ± 2.5	57 ± 8	52 ± 9	60 ± 11	> 30
SI	–	–	11.3 ± 2.1	9.1 ± 2.0	7.8 ± 1.8	47 ± 10	26 ± 12	18 ± 8	18
NS	Sulphinpyrazone	20	19.5 ± 2.9	19.2 ± 2.3	18.6 ± 2.4	50 ± 7	53 ± 5	57 ± 12	> 30
SI	Sulphinpyrazone	1	16.7 ± 2.7*	14.5 ± 2.3*	11.2 ± 2.0	49 ± 10	40 ± 13	32 ± 6*	> 30
SI	Sulphinpyrazone	10	19.4 ± 2.7*	18.7 ± 2.4*	14.3 ± 1.9*	53 ± 12	54 ± 8*	43 ± 6*	> 30
SI	Sulphinpyrazone	20	19.0 ± 2.6*	17.9 ± 2.5*	14.1 ± 2.3*	55 ± 6	61 ± 12*	42 ± 10*	> 30
NS	Indomethacin	20	18.5 ± 2.8	17.0 ± 2.7	19.4 ± 2.2	54 ± 11	58 ± 8	50 ± 11	> 30
SI	Indomethacin	1	11.6 ± 2.2	10.3 ± 2.1	8.0 ± 1.6	51 ± 10	30 ± 7	19 ± 6	21
SI	Indomethacin	10	14.2 ± 2.3*	13.4 ± 2.6*	11.9 ± 2.0*	52 ± 9	38 ± 12	26 ± 8	24
SI	Indomethacin	20	14.0 ± 2.6	12.6 ± 2.7	10.8 ± 2.0	45 ± 7	40 ± 10	29 ± 5*	25
NS	Verapamil	0.5	19.5 ± 2.0	19.3 ± 2.8	20.1 ± 1.9	52 ± 5	50 ± 6	49 ± 12	> 30
SI	Verapamil	0.5	17.7 ± 1.8	15.6 ± 2.6*	12.5 ± 1.2*	58 ± 8	49 ± 8*	51 ± 14*	> 30

There were between 10 and 30 observations in each treatment group. *Indicates a statistically significant difference ($P < 0.05$) from the corresponding value in the group exposed to SI in the absence of a drug. Measurements of right atrial action potential duration measured at 60% repolarization (APD) and rate of conduction (ROC) were made at 1 min intervals throughout the experiments, and the values quoted (mean ± s.e.) were obtained after the listed duration of exposure to the superfusate (NS = normal superfusate). †Time to inter-atrial block is the mean duration of exposure to the superfusate required to produce total failure of conduction of action potentials from the right to the left atrium.

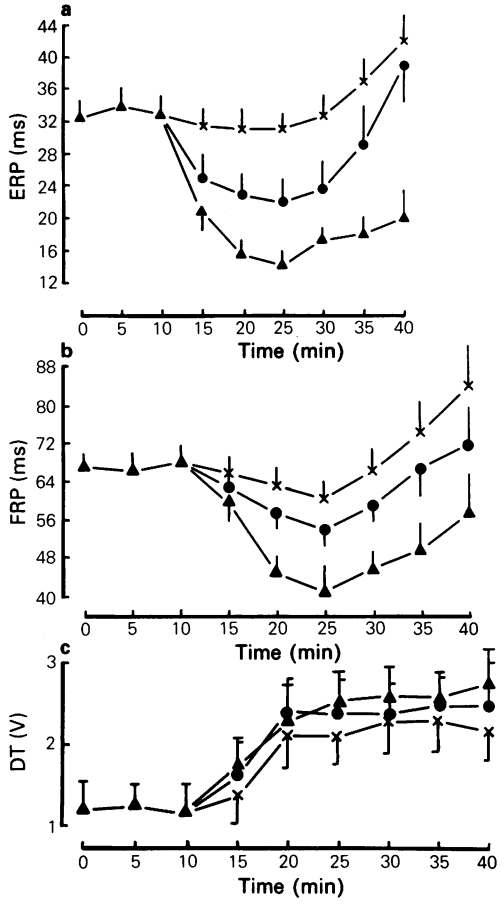

Figure 1 Effects of sulphinpyrazone and of indomethacin on SI-induced changes in the effective refractory period (ERP, a), functional refractory period (FRP, b) and diastolic threshold (DT, c). Tissues exposed to normal superfusate (NS) between 0 and 10 min, and to conditions of simulated ischaemia (SI) between 10 and 40 min. Each point represents the mean of between 8 and 30 observations. The superfusate contained no drug (▲), sulphinpyrazone 10 $\mu g\,ml^{-1}$ (×) or indomethacin 10 $\mu g\,ml^{-1}$ (●). Vertical lines represent s.e.

pattern were seen when the KCl concentration or the pH were kept at values chosen for the NS but the superfusate was made up to contain all the other components of SI. It is concluded, therefore, that the high concentration of KCl and the low pH of SI do not contribute greatly to the electrical disturbances produced by SI. Although restoring the glucose concentration during SI to the value prevailing during NS caused less electrical disturbance than was produced by the full simulation (Figure 2), this concentration of glucose was not fully protective. Nevertheless, at twice the concentration present in NS, glucose fully protected against the SI-induced shortening of ERP (Figure 2).

Omitting magnesium from the superfusate for 30 min during either SI or exposure to otherwise NS, produced slight and statistically insignificant changes in DT, APD, ROC and refractory periods. In contrast, lowering the calcium concentration from 2 mM to 0.5 mM, while having insignificant effects on the rise in DT caused by SI, greatly attenuated the associated shortening of APD and refractory periods (Figure 3), and completely prevented the block of action potential conduction between right and left atrium produced by exposure to SI. Reducing the calcium concentration of NS from 2 mM to 0.5 mM slightly but insignificantly reduced the DT but prolonged APD and refractory periods to an appreciable extent (Figure 3).

Effects of sulphinpyrazone, indomethacin and verapamil

At concentrations of between 1 and 20 $\mu g\,ml^{-1}$ sulphinpyrazone had only insignificant effects on the measured electrical parameters during superfusion with NS. In the same concentration range, however, the drug attenuated the slowing of the ROC and the shortening of both APD (Table 1) and refractory periods caused by exposure to SI (Figures 1 and 4), although the rise in DT was unaltered (Figure 1). The protective effects of sulphinpyrazone were concentration related. The lowest effective concentration tested was 1 $\mu g\,ml^{-1}$ with maximal protection at 10 $\mu g\,ml^{-1}$. The protection afforded by a concentration of 20 $\mu g\,ml^{-1}$ was no greater than that given by 10 $\mu g\,ml^{-1}$ (Figure 4 and Table 1).

Indomethacin was less protective than sulphinpyrazone. The lowest effective concentration of indomethacin tested was 10 $\mu g\,ml^{-1}$, with no greater protection afforded by 20 $\mu g\,ml^{-1}$ (Figure 4 and Table 1), based upon SI-induced shortening of ERP. Hypoxia-induced shortening of ERP was only marginally attenuated by indomethacin at 10 $\mu g\,ml^{-1}$ (Figure 5), whereas sulphinpyrazone at the same concentration gave significant protection (Figure 5).

Verapamil (0.5 $\mu g\,ml^{-1}$) protected against the shortening of both APD and refractory periods caused by exposure to SI and inhibited the associated slowing of the ROC (Table 1). In the presence of this concentration of verapamil exposure to SI for 30 min never produced failure of action potential conduction between the two atria (Table 1). Verapamil at this concentration in NS had no effect upon MRD, ERP, ROC or APD and only slightly and insignificantly prolonged the FRP. However, at a concentration of 5 $\mu g\,ml^{-1}$, verapamil in NS significantly slowed the ROC of action potentials and prolonged both the

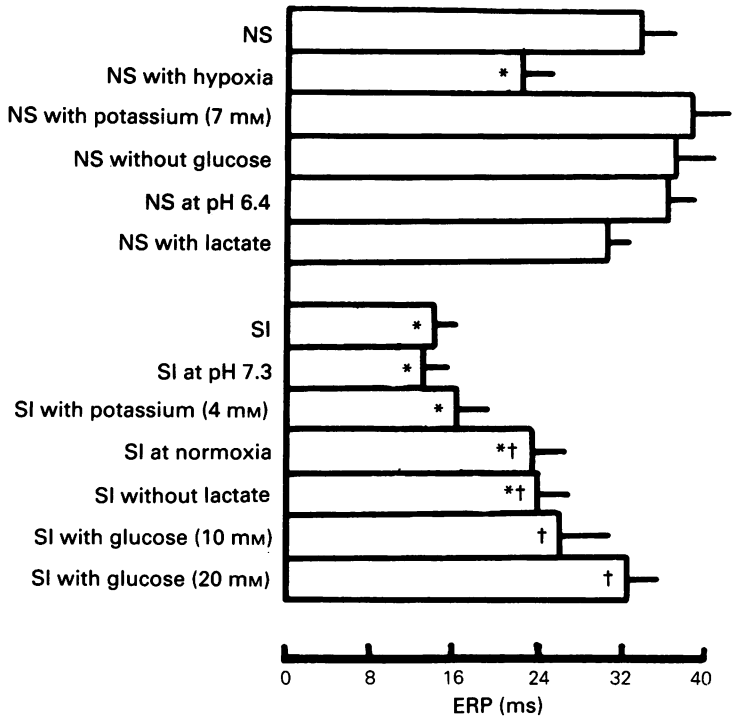

Figure 2 Effect on the effective refractory period (ERP) of exposure for 15 min to conditions of simulated ischaemia (SI) or to its various components. There were between 5 and 11 observations in each treatment group. The horizontal bars at the ends of the columns represent s.e. *Indicates a significant difference ($P < 0.05$) from the normal superfusate (NS) group. †Indicates a significant difference ($P < 0.05$) from the SI group.

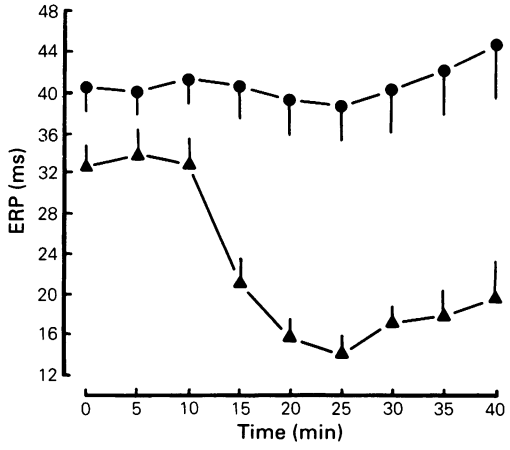

Figure 3 Effect of varying the extracellular calcium concentration on simulated ischaemia (SI)-induced changes in the effective refractory period (ERP). Tissues were exposed to normal superfusate (NS) between 0 and 10 min, and to SI between 10 and 40 min. Each point represents the mean of between 12 and 30 observations. The superfusates contained $CaCl_2$ 2 mM (▲) or 0.5 mM (●). Vertical lines represent s.e.

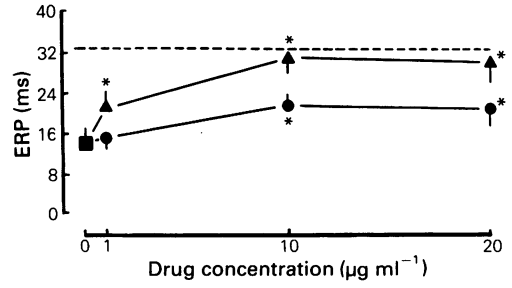

Figure 4 Effect of varying concentrations of sulphinpyrazone or indomethacin on simulated ischaemia (SI)-induced changes in the effective refractory period (ERP). Tissues were exposed to SI for 15 min. Superfusates contained no drug (■), sulphinpyrazone (▲), or indomethacin (●). *Value significantly different ($P < 0.05$) from that obtained in the absence of drugs. Vertical lines represent s.e. The horizontal broken line is the ERP value of tissues in normal superfusate (NS) without drugs.

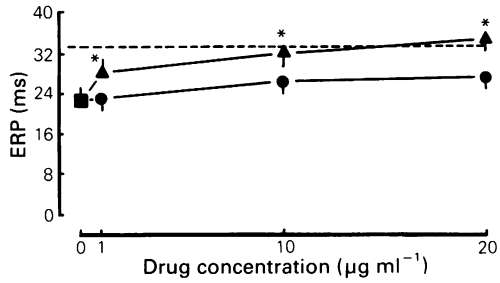

Figure 5 Effect of varying concentrations of sulphinpyrazone or indomethacin on hypoxia-induced changes in the effective refractory period (ERP). Tissues exposed to hypoxia in normal superfusate (NS) for 15 min. Superfusates contained no drug (■), sulphinpyrazone (▲) or indomethacin (●). *Value significantly different ($P < 0.05$) from that obtained in the absence of drugs. Vertical lines represent s.e. Each point represents the mean of between 5 and 12 observations. The horizontal broken line is the ERP value of tissues in NS without drugs.

APD and the refractory periods, the FRP more than the ERP.

Discussion

The present experiments have revealed that under conditions of SI *in vitro*, produced by a combination of abnormally raised concentrations of hydrogen, potassium and lactate ions and a deficiency of oxygen and glucose, rat atrial myocardium displays a temporary shortening of refractory periods, a progressive slowing of the ROC of action potentials and a persistent shortening of the APD. These changes are similar to those described in various other species and in several other parts of the myocardium (see Introduction).

Hypoxia was the only component of SI which separately caused electrical effects that resembled those produced by the full SI. Clearly, however, other components of SI contributed to the observed electrical effects of this type of injury since hypoxia alone produced less marked effects than those produced by full SI. Many previous investigators have observed a shortening of APD and refractory periods *in vitro* during hypoxia. This was first noted in the rat atrium by Webb & Hollander (1956) and in human papillary muscle by Prasad & Callaghan (1969), the subject having been reviewed by Morena *et al.* (1980) and more recently by Conrad *et al.* (1983).

Lack of glucose, by itself, has been demonstrated to have rather variable electrical effects upon the myocardium. Macloed & Prasad (1969) found shortening of APD and refractory periods in guinea-pig ventricular myocardium, whereas tissues from rabbit and dog hearts were unaffected (Senges *et al.*, 1980). From the present results and those of Webb & Hollander (1956), rat atrium seems to resemble the myocardium of the rabbit and dog more than that of the guinea-pig. The guinea-pig heart is exceptional in other respects, because Kodama *et al.* (1984) found that although SI produced the expected shortening of APD in this species, the associated refractory periods were actually lengthened. Despite the latter results, most previous workers have found that the shortening of APD and refractory periods caused by hypoxia was potentiated by a lack of glucose and overcome by elevating glucose concentrations, particularly elevation to supraphysiological levels (Macloed & Prasad, 1969; Prasad & Callaghan, 1969; Morena *et al.*, 1980; Ballantyne & Davis, 1983; Conrad *et al.*, 1983; Cobb *et al.*, 1985). The protective effect of glucose against the shortening of refractory periods caused by SI in the present experiments, therefore, confirms earlier findings.

Maximal shortening of atrial refractory periods in response to SI in the present experiments occurred after 12 min of exposure to injury. This corresponds closely with the time of the greatest shortening of refractory periods and the time of the appearance of the most severe ventricular tachyarrhythmias in the rat heart *in vivo* after coronary artery occlusion (Northover, 1986). The similar timing of the two sets of electrical events suggests that a similar mechanism operates *in vivo* as *in vitro*.

Several previous workers have shown that in dogs, rabbits and guinea-pigs, ischaemia *in vivo* or SI *in vitro* causes a slowing of the ROC of myocardial action potentials which is progressive with time. The present findings indicate that a similar phenomenon occurs in rats. A combination of shortened refractory periods and a slowed ROC of action potentials occurring in a restricted region of the cardiac wall, is powerfully conducive to re-entry arrhythmias (Northover, 1986).

Sulphinpyrazone in the present experiments protected against the shortening of APD and refractory periods and against the slowing of the ROC of action potentials during exposure to SI. This confirms the preliminary results of Iansmith *et al.* (1979) who studied, *in vitro*, the myocardial response to an abnormally low extracellular pH. The protective effect of sulphinpyrazone in the present experiments was graded at concentrations of 1 and 10 $\mu g\,ml^{-1}$. Concentrations of the drug between 0.1 and 1 $\mu g\,ml^{-1}$ have been found by Karmazyn (1984) to protect rat hearts *in vitro* against some of the biochemical consequences of ischaemia. Peak blood plasma concentrations after a 200 mg oral dose to man were shown to reach 13–16 $\mu g\,ml^{-1}$ (Mahony *et al.*, 1983; Pedersen & Fitzgerald, 1985), and doses of 400 mg by mouth gave peak plasma concentrations in excess of 20 $\mu g\,ml^{-1}$

(Rosenkranz *et al.*, 1983). Clearly, therefore, under conditions of ordinary therapeutic use, even allowing for binding of the drug to plasma proteins, an opportunity exists for the drug to exert electrical effects of an anti-arrhythmic type. The same cannot be said for indomethacin, as peak plasma concentrations after a 50 mg oral dose of the drug to man reached only $5\,\mu g\,ml^{-1}$ (Rothermich, 1966), yet the drug was much less effective than sulphinpyrazone in the present experiments in preventing the electrical consequences of SI, with a minimum effective concentration of $10\,\mu g\,ml^{-1}$. Moreover, indomethacin was much less effective than sulphinpyrazone *in vivo* in preventing episodes of ventricular tachyarrhythmia after coronary artery occlusion in the rat and the associated shortening of myocardial refractory periods (Northover, 1986).

Verapamil in the present experiments inhibited both the SI-induced slowing of the ROC of action potentials and the SI-induced shortening of both the APD and the refractory periods. This confirms earlier findings, albeit in other species (Nakaya *et al.*, 1981; 1982; Kimura *et al.*, 1982; 1983; Gettes *et al.*, 1985). Since verapamil blocks the entry of calcium ions via the sarcolemmal slow-channels, this raises the question of the extent to which alterations in myocardial calcium metabolism are responsible for the electrical changes observed during exposure to SI. The shortening of myocardial APD and refractory periods and the slowing of the ROC of action potentials produced by SI in the present experiments were shown to depend upon the concentration of calcium in the superfusate. Previous workers have suggested that calcium ions are involved in the shortening of myocardial APD and refractory periods during both hypoxia and SI (Wojtczak, 1979; Conrad *et al.*, 1983; Kimura *et al.*, 1983; Fitzpatrick & Karmazyn, 1984). Raising the concentration of calcium in the perfusate of isolated hearts from the rat also aggravated the ventricular tachyarrhythmias produced by coronary artery ligation (Daugherty & Woodward, 1981). The mechanisms whereby calcium ions promote shortening of APD and refractory periods, however, are still uncertain. Vleugels *et al.* (1980), using feline voltage-clamped ventricular myocardium, showed that during hypoxia the concentration of calcium ions in the cytoplasm rose. The greater the availability of intracellular calcium ions the greater was the conductance of the sarcolemma to potassium ions. The greater the conductance of the plasma membrane to potassium the earlier the repolarization occurs during an action potential and the shorter the APD. Conrad *et al.* (1983) found evidence that this mechanism operates in the rat heart during hypoxia. At the same time, but independently of effects on potassium ion conductance and APD, a raised concentration of cytoplasmic calcium shortens the time constant for re-activation of the sodium ion channels in the sarcolemma, and thus shortens refractory periods. So far, the latter process has not been studied in rat hearts, but it is well established in the hearts of several other species (Gettes & Reuter, 1974).

The slowing of the ROC of myocardial action potentials during ischaemia *in vivo* and during SI *in vitro* has been attributed, by many previous workers, to alterations in cellular calcium metabolism (Wojtczak, 1979; Nakaya *et al.*, 1980; 1981; 1982; Kimura *et al.*, 1982; 1983; Gettes *et al.*, 1985). It is well established that conduction of action potentials through the myocardium depends upon a selective flow of ionic current through specialised regions of low electrical resistance between adjacent cells (DeMello, 1982). These specialized regions of low electrical resistance are disrupted, lost or occluded when the cytoplasmic calcium concentration rises (DeMello, 1975), as it does during ischaemia, thus slowing the ROC of the action potential. Several groups of workers have demonstrated that slow channel calcium entry blockers of various chemical types (Nakaya *et al.*, 1980; 1981; 1982; Kimura *et al.*, 1982; 1983; Gettes *et al.*, 1985), as well as certain intracellular calcium antagonists (Anno *et al.*, 1986), inhibit the slowing of the ROC of action potentials during exposure of the myocardium to ischaemia or SI. The ability of verapamil in the present experiments, therefore, to inhibit SI-induced slowing of the ROC extends to the rat the findings in other species.

In conclusion, the rat atrium exposed *in vitro* to SI offers an opportunity to study the mechanism of action of anti-arrhythmic drugs. The extent to which these drugs exert their protective effects by altering cellular calcium ion metabolism warrants further study.

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